

## Sub-culture of a Positive EZTest® Biological Indicator

If you desire to sub-culture a positive EZTest biological indicator follow this procedure:

Immediately upon observing that the unit has turned positive (as evidenced by a color change or turbidity), subculture the unit. Waiting until the full incubation time is complete could inhibit the subsequent culturing of these vegetative cells. The vegetative cells produce acidic by-products when they metabolize the carbohydrates in the media. These waste products are what change the pH in the EZTest unit from purple to yellow or red to yellow. Prolonged exposure to this acidic environment will eventually kill all of the vegetative cells.

Note: Sporocidal agents such as bleach or hydrogen peroxide or even IPA should not be used to disinfect the external surfaces of the EZTest unit at any time. Exposure of the unit to anything that would harm vegetative cells is not recommended.

1. In a clean environment (hood or laminar flow bench) and using aseptic technique, carefully remove the EZTest cap and filter paper.
2. Using a 1.0 mL pipette or micropipette with a long tip, extract some of the liquid from the unit. The glass fragments at the bottom of the unit will impede this process. Extract as much of the liquid as possible.
3. Dispense this liquid onto a Trypticase Soy Agar (TSA) plate. Using accepted microbiological culturing techniques spread the liquid evenly across the agar.
4. Incubate plate at 55-60°C for *Geobacillus stearothermophilus* (EZTest Steam) or 30-35°C for *Bacillus atrophaeus* (EZTest gas). Incubate plates for at least 24 hours.
5. Perform appropriate biochemical tests on the colonies from the TSA plates in order to confirm microorganism identity (see table below).

	Methyl Red -Vogues Proskauer (MRVP)	Catalase	Acid production in the presence of carbohydrate source	Growth at 65°C	Dextrose Agar	Tyrosine Agar	Dextrose Starch Agar
<i>Geobacillus stearothermophilus</i>	Neg.	Neg. or weak Pos.	Pos.	Pos.	N/A	N/A	N/A
<i>Bacillus atrophaeus</i>	Pos.	Pos.	Pos.	N/A	Orange pigment	Black pigment *	Pos. (hydrolysis)

\* 5 to 7 days incubation required to observe black pigment

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